Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Comprehensive and Advanced Pharmacology

Journal homepage: www.ijcap.in

Original Research Article

Quantification of the analgesic effect of *Ximenia americana* bark extracts and involvement of opioid receptors

Yacouba Adebo Adehouni[1]*, Amara Kamagate[2], Paul Maomy[3], Sylvain Landry Kouakou[1], Généviève Irie-N'guessan[1], N'doua Gisele Kouakou-Siransy[1]

[1]Laboratory of Pharmacology, Hospital Pharmacy, Human Physiology, Semiology, Faculty of Pharmaceutical and Biological Sciences, Félix Houphouët-Boigny University, Abidjan-Cocody, Ivory Coast

[2]Dept. of Pharmacy, Treichville University Hospital, Ivory Coast

[3]Dept. of Pharmacy, Gamal Abdel Nasser University, Conakry, Guinea

Abstract

Background: Pain is a common symptom requiring timely treatment. This study aimed to quantify the analgesic potential of a decoctate (DXA) and a hydroethanol extract (EHEXA) of *Ximenia americana*, and also to assess the role of opioid receptors.

Materials and Methods: Analgesic effects were measured using Writhing and formaldehyde tests, and the mechanism was investigated by blocking opioid receptors. Efficacy (Emax) and potency (ED_{50}) were the main evaluted pharmacodynamic parameters.

Results: In writhing test, both extracts reduced dose-dependently abdominal contortions for up to 90 minutes, with Emax values of 100%. ED_{50} ranged from 2.84 -1.60 mg/kg (DXA), 7.94 - 0.6 mg/kg (EHEXA), 19.05 - 16.50 mg/kg (paracetamol), and 2.81-3.16 mg/kg (tramadol).

In formaldehyde test, both extracts reduced again dose-dependently neurogenic and inflammatory pain. E_{max} values were 42% and 55% for neurogenic pain, and 65% and 82% for inflammatory pain (DXA and EHEXA, respectively). Potency (ED_{50}) values were around 6 - 7.5 mg/kg of *Ximenia americana*.

Administration of naloxone (Opioid receptor antagonist) inhibited the analgesic effect of the extracts, with a reduction of 23 to 74% for DXA and 42 to 84% for EHEXA from 30 minutes to 90 minutes after, indicating opioid receptor involvement.

Conclusion: *Ximenia americana* extracts showed analgesic effects comparable to reference drugs (paracetamol, tramadol, ketoprofen) and may act as partial opioid receptor agonists at doses ≥10 mg/kg, supporting their potential use in managing mild to moderate pain.

Keywords: Analgesics, Pharmacodynamic parameters, Mechanisms of action, Ximenia americana

Received: 07-05-2025; **Accepted:** 10-06-2025; **Available Online**: 23-07-2025

1. Introduction

Pain, a complex response to tissue damage,[1] represents a major therapeutic challenge, particularly for developing countries where traditional medicine is widely used.[2] WHO estimates that more than 80% of Africans use traditional remedies.[3] *Ximenia americana* (Olacaceae) is among traditionals plants used for pain relief, although its pharmacological properties remain underexplored.[4,5]

A preliminary study conducted by our team showed that decoctate (DXA) and hydroethanol (EHEXA) extracts of *Ximenia americana* inhibited pain.[6] Other studies using different types of extracts (methanolic, ethanolic, aqueous) corroborate its analgesic activity,[3,7-9] but few have quantified this effect using pharmacodynamic parameters.

Most existing research on *Ximemia americana* reports short-term effects (≤ 30 min) and uses single-dose protocols.[3,7,10,11] Several approaches have been put forward to explain the mechanism of action, but in these studies a single dose is used.[11] This study aimed to evaluate effect of *Ximemia americana extracts* (DXA and EHEXA) in acute pain models over a 90-minute period, and study their interactions with opioid receptors.

*Corresponding author: Yacouba Adebo Adehouni
Email: yadehouni@gmail.com

https://doi.org/10.18231/j.ijcaap.2025.015

2. Materials and Methods

2.1. Plant material

Ximenia americana trunk bark was collected in Tiebila (Poro region, Côte d'Ivoire) and authenticated at the National Floristic Center, Félix Houphouët-Boigny University (herbarium no. UCJ013289).

2.2. Animal equipement

Swiss mice (20–30 g) and *Wistar* rats (150–200 g) from the animal facility of Félix Houphouët-Boigny University were used. Animals were housed under standard conditions (20–25 °C, 12 h light/dark), fasted for 4 hours before testing, with water provided ad libitum, following OECD guidelines.[12]

2.2.1. Solvents and chemicals

1. Paracetamol 500 mg tablet from SANOFI Laboratory (Doliprane®)
2. Tramadol 50 mg capsule from ACINO Laboratory (Trabar™-50)
3. Acetic acid 100% from PROLABO Laboratory
4. NaCl 0.9% (500 ml) from Pharmivoire Nouvelle Laboratory
5. Formaldehyde 1%
6. Distilled water
7. Carrageenan
8. Profenid® 100 mg tablet (Aventis Pharma)
9. Naloxone 0,4 mg/ml solution for injection from AGUETTANT

2.3. Preparation of extracts

Ximenia americana bark was cut, dried for 2 weeks at room temperature in the dark, then ground into a 1 mm powder and stored in a sealed container.[6]

2.3.1. Preparation of the decoction of Ximenia americana L. trunk bark

Following Boolamou et *al*. (2015), 200 g of bark powder was boiled in 2 L of distilled water for 10 min, filtered (cotton wool, then Whatman No. 3), and dried at 50°C for 72 h to obtain the aqueous extract.[13]

2.3.2. Preparation of the hydroethanolic extract of Ximenia americana L. trunk bark

Two hundred grams of dry bark powder were macerated for 24 h in 2 L of 70:30 ethanol–water at room temperature. The filtrate (1,455 mL) was filtered and dried at 50 °C for 72 h. The dry extract was stored at 4 °C for further use.[14]

2.4. Quantification of secondary metabolites

2.4.1. Determination of total phenolic content

Total phenolic content was measured by the Folin-Ciocalteu spectrophotometric method, following Singleton and Rossi (1965). Extracts were mixed with Folin reagent and 7.5% sodium carbonate, incubated for 30 min, and absorbance was read at 765 nm. Quantification used a gallic acid standard curve (0–1000 µg/mL).[15]

2.4.2 Determination of Total Flavonoid Content

Flavonoids were quantified spectrophotometrically via complex formation with $AlCl_3$. Extracts were mixed with 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH, then absorbance was measured at 510 nm. Quantification used a quercetin standard curve (0–1000 µg/mL).[15]

2.4.3 Alkaloid ouantification

Alkaloids were quantified spectrophotometrically using Dragendorff's reagent under acidic conditions (pH 2–2.5). After centrifugation, washing, and dissolution in nitric acid, thiourea complexation was measured at 435 nm. Atropine was used as the standard (0–1 mg/mL), with results expressed as atropine equivalents per 100 g or 100 mL.[16]

2.5. Pharmacology test activities

2.5.1 Dorso-abdominal writhing test induced by 1% acetic acid

The method used was that described by Zimmermann.[17]

Mice were divided into 23 groups of five (5) mice each:

1. Group 1: Negative control group treated orally with physiological water.
2. Group 2: Group treated orally with paracetamol at 100 mg/kg body weight (bw).
3. Group 3: Group treated orally with tramadol at 25 mg/kg bw
4. Groups 4 to 13: Experimental groups receiving a range dose of *Ximenia americana* decoction.
5. Groups 14 to 23: Experimental groups receiving a range dose of hydroethanolic extract of *Ximenia americana.*

2.5.1.1. Expression of results[17]

Mean (M) writhing responses were calculated for each group. Pain inhibition (%) was determined by comparing treated groups (extracts, paracetamol, tramadol) to the 0.9% NaCl control, using the following formula:

$$\% \text{ Inhibition} = \frac{(M.\,control\ group\ writhing - M.\,treated\ group\ writhing)}{M.\,control\ group\ writhing} x100$$

A significant reduction in the mean number of writhing responses compared to the control group is considered an analgesic response.

2.5.2. Formaldehyde-induced paw irritation test in rats

Formaldehyde test assesses pain by inducing a biphasic response: an early nociceptive phase (0–10 min) and a late inflammatory phase (15–60 min). Pain behavior

(licking/flinching) is measured to evaluate analgesic efficacy.[18,19]

Rats were divided into 23 groups of five (5) mice each:

1. Group 1: Negative control group treated orally with physiological water.
2. Group 2: Group treated orally with paracetamol at 100 mg/kg body weight (bw).
3. Group 3: Group treated orally with tramadol at 25 mg/kg BW
4. Group 4: Group treated orally with acetic salicylic acid at 100 mg/kg bw
5. Groups 5 to 13: Experimental groups receiving a range dose of ketoprofene (IP administration)
6. Groups 14 to 22: Experimental groups receiving a range dose of *Ximenia americana decoction* (orally administration)
7. Groups 23 to 31: Experimental groups receiving a range dose of hydroethanolic extract of *Ximenia americana*, administered orally

2.5.2.1. Expression of results:[18,19]

The percentage of inflammation inhibition was estimated using the following formula:

$$\% \text{ Inhibition} = \frac{(Licking\ time\ control\ group - licking\ time\ treated\ group)}{Licking\ time\ control\ group} x100$$

2.5.3. Testing involvement opioid receptors in analgesic effect of Ximenia americana extracts.

Opioid involvement was assessed via pharmacological antagonism using naloxone (2 mg/kg, i.p.) administered 15 min before extract treatment. The 1% acetic acid writhing test was conducted 30 min later, with contortions recorded over three 10-minute periods, separated by 20-minute intervals. Mice were divided into 17 groups of five.[20-22]

Mice were divided into 17 groups of five (5) mice each:

1. Group 1: Negative control group treated orally with physiological water and naloxone 2 mg/kg intraperitoneally.
2. Groups 2 to 3: Group treated intraperitoneally with naloxone 2 mg/kg and orally with tramadol at doses of 10 and 25 mg/kg PC.
3. Groups 4 to 10: Experimental groups treated intraperitoneally with naloxone 2 mg/kg and orally with a range dose of *Ximenia americana* decoction.
4. Groups 11 to 17: Experimental groups receiving intraperitoneal administration of naloxone 2 mg/kg and a range dose of hydroethanolic extract of *Ximenia americana,* orally administration.

2.6. Ethical considerations

Animal experiments followed ethical guidelines (e.g., IACUC or EU regulations) and were approved by [institution/committee]. Efforts were made to minimize suffering and reduce animal use, adhering to the 3Rs (Replacement, Reduction, Refinement). The study required animals to investigate pharmacological effects.[23]

2.7. Data treatment and analysis

Data were entered in Excel 2016 and analyzed using GraphPad Prism 9.3.0. Results are expressed as mean ± SD. Mean comparisons were made using the *Kruskal-Wallis* test ($\alpha = 0.05$).

3. Results

3.1. Determination of some secondary metabolites in Ximenia americana bark extracts

Table 1: Results of the determination of alkaloids, polyphenols and flavonoids in *Ximenia americana* extracts

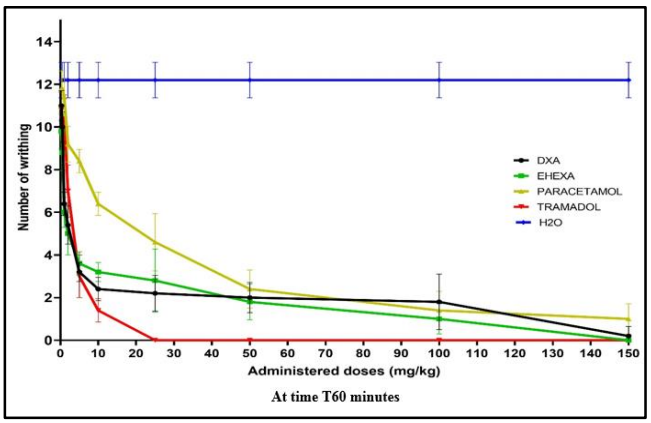
Extracts	Alkaloïds	Polyphenols	Flavonoïds
	C(mgEAT/g Ext)	C(mgEAG/g Ext)	C(mgEQ/g Ext)
EHEXA	0,13±0,015	3,45±0,12	319,5±60,67
DXA	0,08±0,01	3,24±0,01	170,83±09,78

EAT: Atropine Equivalent; EAG: Gallic Acid Equivalent; EQ: Quercetin Equivalent

Table 1 shows EHEXA had slightly higher alkaloids (0.13±0.015 vs 0.08±0.01 mg EAT/g) and similar polyphenols (3.45±0.12 vs 3.24±0.01 mgEAG/g) compared to DXA. However, EHEXA contained significantly more flavonoids (319.5±60.67 vs 170.83±9.78 mgEQ/g).

3.2. Dorso-abdominal writhing test induced by acetic acid 1%

3.2.1. Analgesic potentiel



Kruskal-wallis test: Values expressed as mean standard deviation; risk α=5%; Significant difference *p < 0.05; ** p < 0.01; *** or **** p < 0.001; of extract compared with H2O negative control group.

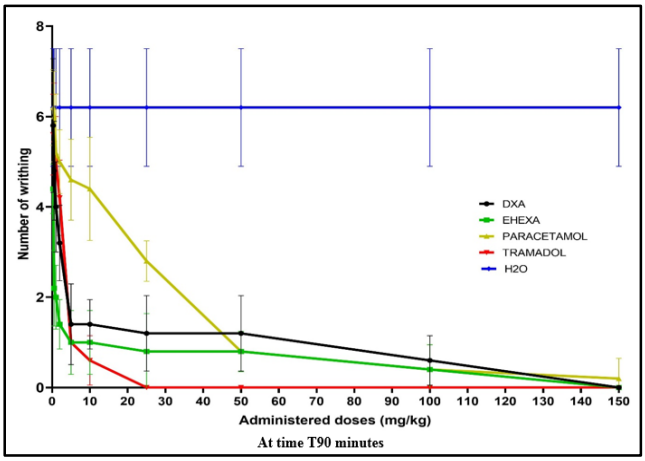
Figure 1: Effect of *Ximenia americana* extracts on contortions at T 60 minutes

Table 2: Writhing inhibition at t 60 minutes

DOSE (mg/kg)	DXA	EHEXA	Tramadol	Paracetamol
0,25	10%	19%	11%	3%
0,5	18%	28%	15%	3%
1	47%	51%	15%	6%
2	55%	59%	43%	24%
5	73%	70%	75%	31%
10	80%	74%	88%	48%
25	82%	76%	100%	62%
50	84%	85%	100%	80%
100	85%	92%	100%	88%
150	100%	100%	100%	92%

Table 2 shows the percentage inhibition of abdominal writhing in mice 60 minutes after substance administration.

At 60 minutes, both extracts showed dose-dependent analgesia ($p<0.05$ at ≥2.5 mg/kg). EHEXA reduced contortions from 19% (0.25 mg/kg) to 100% (150 mg/kg), while DXA ranged from 10% to 98%. Their maximal effects matched reference drugs (tramadol 100% at 25 mg/kg; paracetamol 88%), though requiring higher doses (50-150 mg/kg). Activity profiles were similar between extracts.



Kruskal-wallis test: Values expressed as mean standard deviation; risk α=5%; Significant difference * $p < 0.05$; ** $p < 0.01$; *** or **** $p < 0.001$; tramadol compared with H2O negative control group.

Figure 2: Effect of *Ximenia americana* extracts on contortions at T 90 minutes

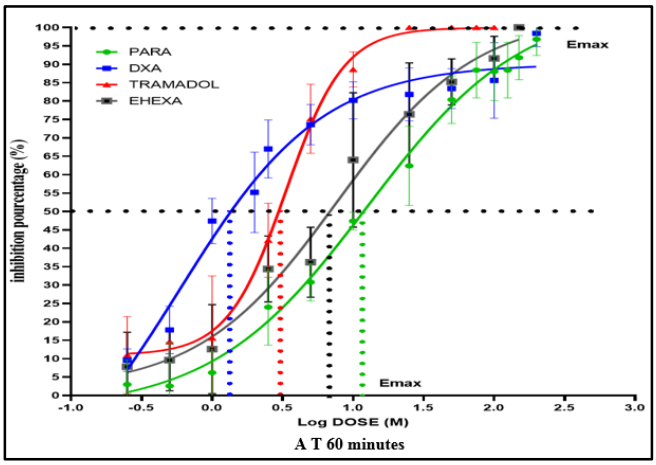
At T90, both extracts showed dose-dependent analgesia, EHEXA: 33% reduction at 0.25 mg/kg → 100% at 150 mg/kg (significant at ≥1 mg/kg, $p<0.05$); DXA: 7% at 0.25 mg/kg → 100% at 150 mg/kg (significant at ≥2.5 mg/kg, $p<0.05$). Both matched reference drugs' efficacy (paracetamol 93%, tramadol 100%) but required higher doses (2.5-150 mg/kg vs tramadol's 25 mg/kg for full effect).

Table 3: Writhing inhibition at t 90 minutes

DOSE (mg/kg)	DXA	EHEXA	Tramadol	Paracetamol
0,25	7%	35%	9%	0%
0,5	23%	64%	13%	3%
1	34%	68%	15%	16%
2	49%	76%	31%	18%
5	77%	84%	83%	25%
10	76%	82%	90%	29%
25	80%	86%	100%	54%
50	79%	87%	100%	86%
100	91%	93%	100%	93%
150	100%	100%	100%	98%

Table 3 shows the percentage inhibition of abdominal writhing in mice 90 minutes after substance administration.

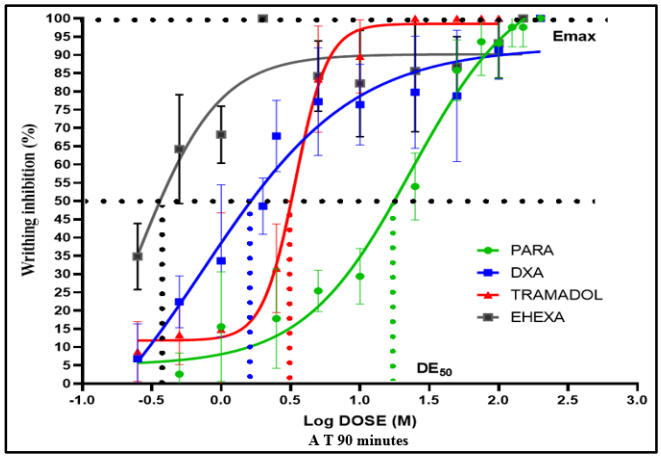
3.2.2. Determination of pharmacodynamic parameters (efficacy-potency)



Norlin-fit test: Contortion inhibition rate; Values expressed as mean deviation. Significant difference in inhibition of extracts (DXA ($p=0.024$) and EHEXA ($p=0.024$) and tramadol ($p=0.023$) compared with paracetamol. Non-significant difference between tramadol-EHEXA ($p=0.001$) and tramadol-DXA ($p=0.053$).

Figure 3: Dose-percentage for inhibition curve of *Ximenia americana* extracts at T 60 minutes.

All compounds (DXA, EHEXA, paracetamol and tramadol) showed identical maximum efficacy (Emax=100%) at T60. ED_{50} comparisons revealed: DXA (1.60 mg/kg,) was 2 times more potent than tramadol (3.09 mg/kg, $p=0.053$) and 10 times more potent than paracetamol (12.58 mg/kg, $p=0.024$). EHEXA (7.07 mg/kg) was 2 times less potent than tramadol ($p=0.087$) but 2 times more potent than paracetamol ($p=0.024$). DXA was significantly more potent than EHEXA ($p<0.05$). Tramadol showed intermediate potency: stronger than paracetamol ($p=0.023$) but comparable to DXA ($p=0.053$) and superior to EHEXA ($p=0.001$).

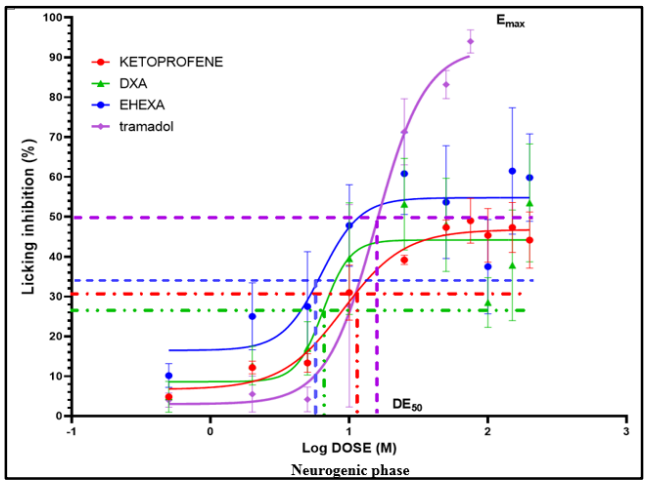


Norlin-fit variant Anderson-Darling test: Contortion inhibition rate; Values expressed as mean deviation. Significant difference in inhibition of extract and tramadol compared with paracetamol (extract-paracetamol: p = 0.0046; tramadol-paracetamol: p < 0.0001). Significant difference tramadol-extract p = 0.0237

Figure 4: Dose-percentage inhibition curve for *Ximenia americana* extracts at T 90 minutes

Both *Ximenia americana* extracts showed comparable maximum efficacy (E_{max}=90%) to reference drugs (paracetamol and tramadol, E_{max}=100%) at T90 minutes. ED_{50} analysis revealed: DXA (1.70 mg/kg) 10 times more potent than paracetamol (p=0.021) and 2 times more potent than tramadol (p=0.087, NS). EHEXA (0.60 mg/kg) 20 times more potent than paracetamol and 5 times more potent than tramadol (both p=0.001). EHEXA was 2.5× more potent than DXA (p<0.05).

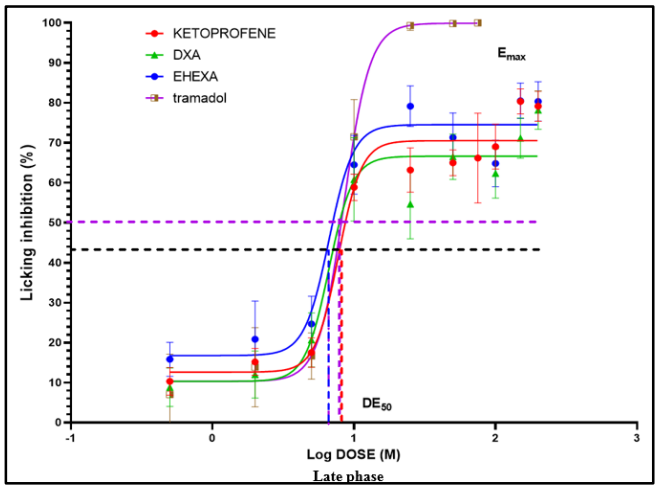
3.3. Formaldehyde-induced paw irritation test in rats



Norlin-fit test: Inhibition rate of the licking time of the right hind paw; Values expressed as mean deviation. Non-significant difference in inhibition of neurogenic pain by extracts (DXA (p=0.001); EHEXA (p=0.0001) compared with ketoprofen and DXA-EHEXA (p=0.910).

Figure 5: Dose-percentage for inhibition curve of *Ximenia americana* extracts during the neurogenic phase of the formaldehyde test

During neurogenic phase of formaldehyde test, Efficacy (E_{max}) of Tramadol (94%) > EHEXA (55%) (p=0.001)> ketoprofen (45%) > DXA (42%). Potency (ED_{50}): EHEXA (6.28 mg/kg) ≈ DXA (6.48 mg/kg) (p=0.901, ns)> ketoprofen (9.04 mg/kg) > tramadol (15.47 mg/kg)



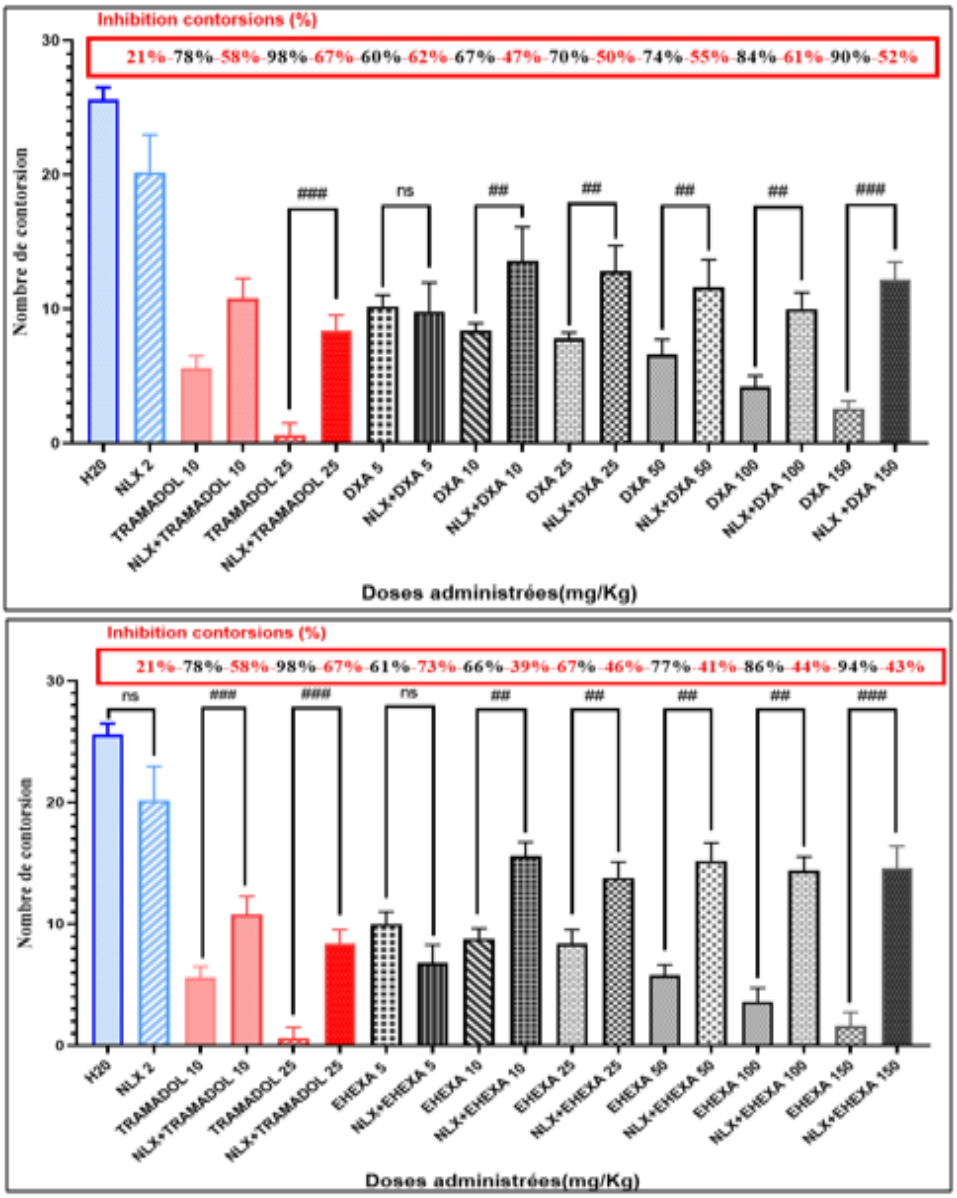
Norlin-fit test: Inhibition rate of the licking time of the right hind paw; Values expressed as mean deviation. Non-significant difference in inhibition of inflammatory pain by extracts (DXA (p=0.001) and EHEXA (p=0.001) compared with tramadol and DXA-EHEXA (p=0.910).

Figure 6: Dose-percentage curve for inhibition of *Ximenia americana* extracts during the inflammatory phase of the formaldehyde test.

Anti-inflammatory effect (late phase) showed Efficacy (E_{max}) of Tramadol (100%) (p=0.001)> EHEXA (82%) > ketoprofen (74%) > DXA (65%). Potency (ED_{50}): DXA (6.65 mg/kg) ≈ EHEXA (7.50 mg/kg) ≈ ketoprofen (7.75 mg/kg) < tramadol (8.52 mg/kg).

3.4. Impact of Naloxone on Ximenia americana extracts anti-nociceptive properties

Ximenia americana extracts (10-150 mg/kg) significant inhibited contortions compared with control (p≤0.05) with pain reducing from 60 to 94%. Co-administration Naloxone (NLX, 2 mg/kg) + *Ximenia americana* extracts (5-150 mg/kg) at doses >10 mg/kg reduced analgesia potential to 40-55% (p=0.001 vs extracts alone).



Kruskal-wallis test: Values expressed as mean standard deviation; risk α=5%; Significant difference $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$; $^{\#\#\#}$ or $^{\#\#\#\#}$ $p < 0.001$; tramadol compared with H2O negative control group.

Figure 7: Effect of naloxone on the action of *Ximenia americana* extracts at T 30 min

4. Discussion

The objective of this study was to quantify analgesic potential of substance using pharmacodynamic parameters, efficacy and pharmacological potency. These parameters are tools used to compare several drug substances with each other.[24-26] Subsequently, involvement of opioid receptors was investigated.

In our previous study, we demonstrated analgesic potential of *Ximenia americana* extracts 30 minutes after induction of pain by acetic acid.[6] In order to ascertain whether this analgesic effect was transient or enduring over time, evaluation was extended to 60 and 90 minutes following pain induction. Pharmacodynamic parameters were determined in order to quantify this effect. Subsequent to results obtained on subject of analgesic effects, a search was initiated for a mechanism of action that was mediated by opioid receptors.

Analgesic effects of DXA and EHEXA were sustained from 60 minutes to 90 minutes, with a dose-dependent inhibition of abdominal contortions at T60 minutes and T90 minutes.

With regard to pharmacodynamic parameters, DXA and EHEXA exhibited pharmacological efficacy similar to that of reference drugs (paracetamol and tramadol) (E_{max}= 100% at T90). However, with regard to pharmacological potency, DXA demonstrated consistent potency over 60–90-minute period (ED_{50}: 1.60 mg/kg at T60 minutes versus 1.70 mg/kg at T90 minutes). In contrast, EHEXA exhibited a 10-fold increase in potency from T60 minutes (ED_{50} = 7.07 mg/kg) to T90 minutes (ED_{50} = 0.6 mg/kg), indicating an

enhancement in its efficacy over time. However, ED_{50} of EHEXA is not precise because the semi-logarithmic transformation of dose-response curve did not produce a sigmoid.

In comparison with tramadol, DXA exhibited comparable potency at 60 minutes, while EHEXA demonstrated a twofold reduction in potency. Extracts were found to be more potent than paracetamol. However, at 90 minutes, EHEXA (ED_{50}= 0.60 mg/kg) was found to be five times more potent than tramadol (ED_{50}=3.16 mg/kg), while DXA (1.70 mg/kg) was approximately three times more potent.

By superimposing our previous results on those of present study, it was found that pharmacological efficacy of extracts and reference substances remained constant (100%) from 30 minutes to 90 minutes. However, with regard to pharmacological potency, an increase in potency of DXA was observed. ED_{50} of DXA exhibited a range from 2.84 mg/kg at 30 minutes to 1.70 mg/kg at 90 minutes, indicative of 1.6 fold increase in potency. For EHEXA, ED_{50} decreased from 7.94 mg/kg at 30 minutes to 0.60 mg/kg at 90 minutes, indicating a 13-fold increase in potency. In contrast, potency of tramadol and paracetamol remained consistent over the duration of study, ranging from 30 minutes to 90 minutes.

Present findings are in accordance with the research conducted by Konaté Kiessoun *et al*. in Burkina Faso, which demonstrated that analgesic efficacy of polyphenol-rich fractions of aqueous acetone extract of *Ximenia americana* root, administered at a dose of 200 mg/kg, remained consistent until a duration of five hours had elapsed in an acetic acid-induced pain model. [9]

As Writhing test is non-selective for pain, it is possible that our extracts could have activities on pain, inflammation or even spasms.[27,28] As demonstrated in extant literature, both extracts exhibit analgesic potential, which is likely attributable to the inhibition of pain mediators, including prostaglandins, opioid receptors, and simultaneous inhibition of multiple pain signalling pathways.[29-31]

In order to pursue this pharmacological investigation, formaldehyde test was utilised, a method which is widely regarded as the standard for assessing nociception in animals. This approach offers significant advantage of enabling discrimination between neurogenic and inflammatory components of pain.[29,32]

Results of neurogenic phase of formaldehyde test demonstrated dose-dependent analgesic activity of DXA and EHEXA extracts on neurogenic pain. Present study demonstrated that EHEXA was marginally more efficacious than DXA. EHEXA demonstrated a comparable analgesic effect to DXA, exhibiting similar ED_{50} values (6.28 mg/kg for EHEXA versus 6.48 mg/kg for DXA).

Pharmacological potency of both extracts was found to be significantly greater than that of tramadol (ED_{50}=15.47mg/kg, p=0.001). Nevertheless, efficacy of these medications was found to be inferior to that of tramadol. It is therefore hypothesised that extracts of *Ximenia americana* have potential to be developed as new analgesic pharmaceutical agent.

A review of extant scientific literature reveals that *Ximenia americana* possesses significant analgesic potential in context of formaldehyde-induced neurogenic pain.[11,33]

In late phase of formaldehyde test, results obtained demonstrated that of two extracts, EHEXA was more effective than DXA. Ketoprofen utilised as a reference in this study exhibited reduced efficacy in comparison to EHEXA, yet demonstrated superiority over DXA. With regard to their pharmacological potency, no significant difference was observed between extracts (p=0.098). Potency of ketoprofen (ED_{50}=7.747mg/kg), utilised as a reference substance, exhibited no statistically significant variation from that of extract (Keto-DXA p=0.078; Keto-EHEXA p= 0.673). These results suggest that two *Ximenia americana* extracts could therefore be a candidate drug for treating pain of inflammatory origin.

Findings of this study are in accordance with results of several scientific studies which suggest that *Ximenia americana* possesses analgesic properties for inflammatory pain induced by formaldehyde.[3,11,33,34]

In traditional medicine, *Ximenia americana* extracts are utilised for the treatment of joint pain and rheumatism in Ivory Coast,[34] Senegal,[35] and Burkina Faso,[36] toothache in Benin[37] and Tanzania,[38] and simple muscular pain in massage in South Africa[39] and Mali.[40]

As the neurogenic phase of formaldehyde test progresses, there is an observable increase in pharmacological efficacy of DXA and EHEXA, with values rising from 42% to 65% and 55% to 82%, respectively. This phenomenon is indicative of an inflammatory phase transition. Nevertheless, pharmacological potency of extracts remained constant, ranging from 6.48 to 6.65 mg/kg and from 6.28 to 7.50 mg/kg for DXA and EHEXA, respectively.

Observed effects of extracts can be attributed to interaction of mediators from neurogenic (Substance P, CGRP, glutamate) and inflammatory (TNF-α, IL-1β, PGE2) phases with opioid receptors (μ, δ, κ), which modulate pain. μ-Opioid agonists have been shown to inhibit the release of Substance P and pro-inflammatory cytokines, while δ-opioid receptors have been observed to attenuate hyperalgesia. Exogenous opioids such as morphine have also been demonstrated to potentiate anti-inflammatory effects by interfering with COX-2 pathway.[41,42]

It is acknowledged that tramadol exerts its effects, at least in part, through stimulation of opioid receptors.[43,44] In

light of this, an investigation was conducted to ascertain the impact of our extracts on these receptors, given their superior potency in comparison to that of tramadol.

Co-administration of naloxone with extracts resulted in a reduced pharmacological effect for both extracts. However, at a dose of 5 mg/kg, co-administration of naloxone did not result in any change in analgesic effect of two extracts. It was only at doses ranging from 10 to 150 mg/kg that a modification of analgesic effect was observed. Furthermore, it was observed that this inhibition increased over time. For instance, an experiment was conducted in which a dose of 100 mg/kg was administered at T30, T60 and T90 minutes. Results demonstrated a reduction in pharmacological effect of 23%, 47% and 74% for DXA and 42%, 62% and 84% for EHEXA, respectively. These findings provide evidence for the reversible effect of naloxone.

Furthermore, intrinsic (α) activity of extracts on opioid receptors can be quantified.[45] Therefore, on basis that intrinsic activity of tramadol (E_{max}=94%) is equal to 1, it can be hypothesised that the intrinsic activity of DXA and EHEXA is 0.44 and 0.58, respectively, during neurogenic phase of formaldehyde test. Consequently, *Ximenia americana* extracts can be regarded as partial agonists.

These results allow us to hypothesise that analgesic activity of our extracts is mediated in part by opioid receptors. Furthermore, the analgesic effect of this substance, which is mediated by opioid receptors, has been observed to increase over time. The release of active ingredients from the complex is a gradual process, and this is the basis for justification of extract as a complex of active substances.[46]

In contrast to work of Pessoa *et al*, which evaluated mechanism of action of a hydroethanol extract of *Ximenia americana* at a single dose of 100 mg/kg, our work evaluated a dose range from 5 mg/kg to 150 mg/kg.[11]

Consequently, extracts of *Ximenia americana* (DXA and EHEXA) exhibited a dose-dependent response in antinociceptive pathways. Furthermore, DXA and EHEXA demonstrated non-opioid activity at a dose of 5 mg/kg and opioid action from 10 mg/kg. This opioid activity persisted up to a dose of 150 mg/kg.

These results are consistent with those of Pessoa et *al*. who demonstrated that HEXA (100 mg/kg) and morphine significantly reduced nociception time (57.28% and 94.20% respectively, $p<0.0001$), with an effect reversible by naloxone, confirming involvement of the opioid system.[11]

The discrepancy in effect between the DXA and EHEXA extracts can be attributed to their phytochemical composition. Flavonoid assay revealed a substantial discrepancy between the two extracts.

5. Conclusion

Pharmacodynamic analysis showed that *Ximenia americana* extracts had comparable efficacy and potency to reference drugs (tramadol, paracetamol, ketoprofen), with effects lasting 90 minutes like tramadol. Their analgesic action involved opioid receptors at doses ≥10 mg/kg, acting as partial agonists (intrinsic activity <1). These findings suggest their potential as phytomedicines for pain relief via partial opioid agonism.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. International Association for the Study of Pain. *IASP Terminology:* Pain. 2024.
2. Chambers CT, Dola J, Tutelman PR, Langley CL, Parker JA, Cormier BT, et *al*. The prevalence of chronic pain in children and adolescents: A systematic review update and meta-analysis. *Pain*. 2024;165(10):2215–34.
3. Soro TY, Traore F, Sakande J. Activité analgésique de l'extrait aqueux de *Ximenia americana* (Linné) (Olacaceae). Comptes Rendus Biologies. 2009;332(4):371–7.
4. World Health Organization. WHO traditional medicine strategy 2014-2023. Geneva: WHO; 2013 [cited 2024 Jun 20].
5. Organisation Ouest-Africaine de la Santé. Pharmacopée d'Afrique de l'Ouest. 1st ed. Knust-Junction; 2013.
6. Adehouni YA, Maomy P, Kamagate A, Effo KE, Kouakou SL, Djadji ATL. Evaluation of the Analgesic and Anti-Inflammatory Activity of the Decoction and Hydroethanolic Extract of *Ximenia americana* L. (Olacaceae) Bark. *Pharmacol Pharm*. 2025;16(4):105–23.
7. Soro TY, Zahoui OS, Nene-Bi AS, Traore F. Analgesic activity of the fractions of the aqueous extract of *Ximenia americana* (Linné) (Olacaceae). *Int J Pharmacol Toxicol*. 2016;4(1):1–6.
8. Nyangacha R, Mwangi J, Mworia J. Antinociceptive effect of *Pentas lanceolata* and *Ximenia americana* medicinal plants used to treat malaria traditionally in Kenya. *Afr J Pharmacol Ther*. 2016;5(2):87–92.
9. Konaté K, Kiessoun B, Nacoulma OG. Anti-Nociceptive and Anti-Inflammatory Properties of Polyphenol-Rich Fractions of Roots from *Ximenia americana* L., (Olacaceae), in Experimental Mice. *Int J Pharmacol Phytochem Res*. 2018;12(1):281–97.
10. Kenmogne SB, Ngassoum M, Tchatchueng JB, Vardamides JC, Dongmo A. Extraction assistée par micro-ondes de composés analgésiques de la racine de *Ximenia americana* (Olacaceae). *Rev Rech Sci Chim*. 2014;2231:606
11. Pessoa RT, da Silva LYS, Alcântara IS, Silva TM, da Silva ES, da Costa RHS, et *al*. Antinociceptive potential of *Ximenia americana* L. bark extract and caffeic acid: Insights into pain modulation pathways. *Pharmaceuticals*. 2024;17(12):1671.
12. Organisation for Economic Co-operation and Development. OECD guidelines for the testing of chemicals: Good laboratory practice [Internet]. [cited 2025 0 1 04]. Paris; 2018
13. Boolamou B, Lapo A, Camara K, Assane M, Bassene E, Samb A. Activité anti-inflammatoire du décocté aqueux des écorces de racines de *Morinda geminata* DC (Rubiaceae). *Int J Biol Chem Sci*. 2015;8:1871–5.
14. Seo J, Lee S, Elam ML, Johnson SA, Kang J, Arjmandi BH. Study to find the best extraction solvent for use with guava leaves (*Psidium*

guajava L.) for high antioxidant efficacy. *Food Sci Nutr*. 2014;2(2):174–80.
15. Ali-Rachedi F, Meraghni S, Touaibia N, Marniche S. Analyse quantitative des composés phénoliques d'une endémique algérienne *Scabiosa atropurpurea* sub. *Maritima* L. *Bull Soc R Sci Liège*. 2018;87:13–21.
16. Togola S, Konaré MA, Diakité M, Diarra N, Tounkara F, Sanogo R, et al. Evaluation of the content of total alkaloids at different stages of development of *Datura innoxia* Mill, a plant used in traditional medicine in Mali. *Am J Innov Res Appl Sci*. 2023;16(4):112–20.
17. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*. 1983;16(2):109–10.
18. Dubuisson D, Dennis SG. The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain*. 1977;4(2):161–74.
19. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: An evaluation of the method. *Pain*. 1992;51(1):5–17.
20. Mahdian Dehkordi FM, Kaboutari J, Zendehdel M, Javdani M. The antinociceptive effect of artemisinin on the inflammatory pain and role of GABAergic and opioidergic systems. *Korean J Pain*. 2019;32(3):160–7.
21. De Sousa DP. Medicinal essential oils: Chemical, pharmacological and therapeutic aspects. New York: *Nova Science Publishers*; 2012.
22. Hijazi MA, El-Mallah A, Aboul-Ela M, Ellakany A. Evaluation of analgesic activity of *Papaver libanoticum* extract in mice: Involvement of opioids receptors. *Evid Based Complement Alternat Med*. 2017;2017:8935085.
23. D'Amato ME, Baumgartner MR, Haas C, Coble MD. Ethical publication of research on genetics and genomics of biological material: Guidelines and recommendations. *Forensic Sci Int Rep*. 2020;2:100091.
24. Cohen R, Tauzin M, Rybak A, Ouldali N, Grimprel E. Définitions et implications des paramètres pharmacocinétiques-pharmacodynamiques des antibiotiques en pratique clinique pédiatrique. *J Pediatr Pueric*. 2024;37(3):123–8.
25. Kenakin T. Pharmacology in drug discovery and development: Understanding drug response. 2nd ed. Amsterdam: *Elsevier*; 2017.
26. Beaulieu P, Pichette V, Desroches J, du Souich P, editors. Précis de pharmacologie: Du fondamental à la clinique. Montréal: Presses de l'Université de Montréal; 2015.
27. Le Bars D, Pollin B, Plaghki L. Quelle est la validité conceptuelle des tests et modèles animaux de douleurs? *Douleur Analg*. 2012;25(1):2–30.
28. Matricon J, Gelot A, Ardid D. Modèles expérimentaux de douleurs pelvi-périnéales. *Douleur Analg*. 2007;20(3):173–9.
29. Beirith A, Ferreira J, Santos ARS, Calixto JB. Mechanisms underlying the antinociception caused by ethanolic extract obtained from the leaves of *Melissa officinalis* in mice. *J Ethnopharmacol*. 2002;83(1-2):109–13.
30. Derry S, Wiffen PJ, Moore RA. Tramadol for neuropathic pain in adults. *Cochrane Database Syst Rev*. 2017;6:CD012522.
31. Högestätt ED, Jönsson BA, Ermund A, Andersson DA, Björk H, Alexander JP, et al. Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *J Biol Chem*. 2005;280(36):31405-12.
32. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain*. 1987;30(1):103–14.
33. Ntchapda F, Dimo T, Sonfack AS, Nkeng-Efouet PA, Théophile D. Anti-inflammatory and analgesic properties of aqueous bark extract of *Ximenia americana* L. (Olacaceae) in rodent models. BMC *Complement Altern Med*. 2015;15:258.
34. Koné WM, Atindehou KK, Terreaux C, Hostettmann K, Traoré D, Dosso M. Traditional medicine in north Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. *J Ethnopharmacol*. 2004;93(1):43–9.
35. Kerharo J, Adam JG. La pharmacopée sénégalaise traditionnelle. Paris: Vigot; 1974.
36. Nacoulma/Ouédraogo, O.G. (1996) Plantes médicinales et Pratiques médicales Traditionnelles au Burkina Faso: Cas du plateau central T1 & T2. Thèse de l'université Ouaga I Pr Joseph KI-ZERBO, 91-92.
37. Akoègninou A, van der Burg WJ, van der Maesen LJG. Flore analytique du Bénin. Leiden: Backhuys Publishers; 2006.
38. Chhabra SC, Mahunnah RLA, Mshiu EN. Plants used in traditional medicine in Eastern Tanzania. *J Ethnopharmacol*. 1990;29(3):295–323.
39. Van Wyk BE, Oudtshoorn B, Gericke N. Medicinal plants of South Africa. 2nd ed. Pretoria: *Briza Publications*; 2018.
40. Diallo D, Hveem B, Mahmoud MA, Berge G, Paulsen BS, Maiga A. An ethnobotanical survey of herbal drugs of Gourma district, Mali. *J Ethnopharmacol*. 2002;79(1):43–7.
41. Stein C. Opioid receptors. *Annu Rev Med*. 2016;67:433–51.
42. Zöllner C, Stein C. Opioids. *Handb Exp Pharmacol*. 2007;177:31–63.
43. Depiesse M. Représentations du mésusage d'antalgiques opioïdes. Thèse de doctorat en médecine, Université de Strasbourg. Strasbourg 2023.
44. Gauthier M, Buxeraud J, Faure S. Les médicaments à risque d'usage détourné et de dépendance. *Actual Pharm*. 2024;63(635):20–6.
45. Grim TW, Acevedo-Canabal A, Bohn LM, Navarro HA. Methods to quantify ligand efficacy at the mu-opioid receptor. *Methods Mol Biol*. 2020;2201:131–50.
46. Mbikay M. Potentiel thérapeutique des feuilles de *Moringa oleifera* dans l'hyperglycémie chronique et la dyslipidémie: une revue. *Front Pharmacol*. 2012;3:24.

Cite this article: Adehouni YA, Kamagate A, Maomy P, Kouakou SL, Irie-N'guessan G., Kouakou-Siransy NG. Quantification of the analgesic effect of *Ximenia americana* bark extracts and involvement of opioid receptors. *IP Int J Compr Adv Pharmacol*. 2025;10(2):104–112.